Magnetic resonance studies on glutamate dehydrogenase

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SUMMARY

This thesis deals with the binding of substrate, coenzyme and other ligands to the enzyme glutamate dehydrogenase. The major technique used in the investigations was magnetic resonance.

In chapter II the theory of NMR transverse relaxation times of small ligands binding to macromolecules is briefly reviewed. Theoretical equations are derived for several binding mechanisms. It is shown that the application of the NMR technique is often necessary in connection with other measuring methods to give a detailed description of the binding process.

A revision of the theory for longitudinal relaxation times of binding ligands is presented in chapter III. The theory is supported by experimental evidence obtained for α-ketoglutarate binding to glutamate dehydrogenase.

Chapter IV is devoted to the interactions of substrate α-ketoglutarate, coenzymes NAD(P)(H), and effectors of the catalytic activity, with the enzyme. In the studies use is made of the transverse relaxation time of the substrate which is strongly decreased by interaction with the enzyme, and in turn increases again if coenzymes are added to form a ternary complex.

From a quantitative study of these effects thermodynamic equilibrium constants are obtained. Information about dissociation rates becomes also available which is difficult to obtain from other techniques. This kinetic information leads to the conclusion that the binding of reduced coenzyme the binary complex, and the binding of coenzyme and substrate in the ternary complex, are not a simple single step process.

In chapter V we present the results of modification of the enzymes with two kinds of stable paramagnetic nitroxides (spin labels). One of them reacts specifically with a residue (probably lysine 126) located in the active centre.The nuclear relaxation of bound ligands by the paramagnetic group was studied for α-ketoglutarate, the coenzyme NADP and the inhibitor GTP of the enzyme. For all these compounds a binding site close to the spin label was detected; the substrate in particular is located very near to the spin label. The implications of our findings for allosteric theories are discussed.

The binding of manganese as analog for the inhibitory zinc ion is investigated in chapter VI. It turned out that under the conditions used in our NMR and ESR experiments the binding was too weak to produce significant effects.

The binding of ligands to multiprotomeric enzymes is the subject of chapter VII. The present models for cooperative binding are discussed. It will be considered what the implications are of cooperative effects for NMR binding studies.

SAMENVATTING

Enzymen zijn een bepaald soort eiwitmoleculen, die ervoor zorgen dat de zeer vele chemische reakties die in ons lichaam plaatsvinden snel kunnen verlopen. Iedere reaktie heeft weer zijn eigen enzym. Eén van de vele bekende enzymen, glutamaat dehydrogenase genaamd, is het onderwerp van dit proefschrift.

Een van de eigenschappen van een bepaald enzym is, dat het die speciale stoffen die met elkaar moeten reageren (substraten en zgn. coenzymen) eerst aan zich bindt, waarschijnlijk in een zeer speciaal gekonstrueerd gleufje of holletje. Hiermee worden dan waarschijnlijk die stoffen zo ten opzichte van elkaar gericht dat de reaktie snel kan optreden, veel sneller dan wanneer er geen enzym zou zijn.

Dit proefschrift beschrijft studies van dit bindingsproces voornamelijk met behulp van een speciale techniek, de zogenaamde magnetische resonantie. Met deze techniek (waar we niet verder op ingaan) kunnen allerlei stoffen in een oplossing bestudeerd worden. De techniek geeft bij voorbeeld informatie over hoe stevig zo'n stof aan een enzym gebonden zit en hoe snel hij van het enzym molekuul weer terug gaat in de oplossing . Het blijkt onder andere uit het onderzoek,dat dit bindingsproces nog vrij ingewikkeld kan zijn, de binding kan op verschillende manieren plaatsvinden, of verloopt misschien via